Quality Management Plan

and

Quality Assurance Project Plan

for

Shipboard Tests of the

SiCURE Ballast Water Treatment System

developed by

Siemens

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GoConsult

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1. Introduction

Ballast water management systems have to meet the Ballast Water Performance Standard as stipulated in Regulation D-2 (see below) of the IMO Convention for the Control and Management of Ship`s Ballast Water and Sediments. To challenge candidate treatment systems comprehensive and rigorous tests have to be undertaken using both land-based and shipboard trials.

The test requirements are outlined in the IMO Guideline for Approval of Ballast Water Management Systems (G8). Although shipboard tests are required in G8 as part of the approval process of a treatment system, the guidelines lack specific and detailed sampling protocols.

This protocol intends to overcome this shortcoming. The most up-to-date version of the Guideline G8 as agreed by IMO MEPC58 in October 2008 was used to prepare this sampling protocol.

The onboard tests of the SiCURE ballast water treatment system developed by Siemens will be undertaken on the container vessel *COSCO Fortune*. The vessel is fitted with one SiCURE system to match the ballast water flow capacity of the vessel.

The required duration of the test period is at least 6 months and the first tests are planned to start in summer 2012. Two ballast tanks are identified for the tests, i.e. one tank for treated water and another for the control experiments. As required by IMO the tests will be carried out during routine ship operations. While the timing of ship visits is not easily determined, the majority of the tests are likely to take place in Europe.

1.1. IMO Ballast Water Discharge Standard

Regulation D-2 of the Convention stipulates that in order for ships to meet the requirements of the Convention these must discharge:

- Regulation D-2.1:
  - less than 10 viable organisms per cubic meter greater than or equal to 50 micrometers in minimum dimension, and
  - less than 10 viable organisms per millilitre less than 50 micrometers in minimum dimension and greater than or equal to 10 micrometers in minimum dimension, and
- Regulation D-2.2:
  - less than the following concentrations of indicator microbes, as a human health standard:
    - Toxigenic *Vibrio cholerae* (serotypes O1 and O139) with less than 1 Colony Forming Unit (cfu) per 100 millilitres or less than 1 cfu per 1 gramme (wet weight) of zooplankton samples,
    - *Escherichia coli* less than 250 cfu per 100 millilitres, and
    - Intestinal *Enterococci* less than 100 cfu per 100 millilitres.

As a consequence the treatment method must show a capability of reducing the viability of organisms to these standards according to their size. Further, the system must also address the indicator bacteria as referred to in Regulation D-2.

2. Purpose of this protocol

The purpose of this sampling protocol is to prepare and produce a practical standard system for the shipboard efficacy tests of the ballast water treatment system. This protocol

- defines the test requirements for on-board efficacy tests, and
- describes the appropriate sampling strategies and procedures for such tests.

3. Challenge water

Standardised water conditions in shipboard tests as outlined in G8 address minimum numbers of organisms during ballast water uptake to "challenge" the treatment system under consideration. In addition abiotic water parameters need to be documented.

3.1. Abiotic water conditions

The source water for test cycles shall be characterized by measurement of salinity, temperature, particulate organic carbon and total suspended solids[1].

3.2. Organisms

Valid tests are indicated by uptake water, for both the control tank and ballast water to be treated, with viable organism concentrations exceeding 10 times the maximum permitted values in Regulation D-2.1 and control tank viable organism concentrations exceeding the values of Regulation D-2.1 on discharge[2].

This results in a minimum required organism density in the water to be treated and also for the control experiment during **uptake**:

- at least 90 viable organisms per cubic meter greater than or equal to 50 micrometers in minimum dimension, and
- at least 90 viable organisms per millilitre less than 50 micrometers in minimum dimension and greater than or equal to 10 micrometers in minimum dimension
- minimum concentrations of bacteriae are not required.

Upon **discharge** the number of organisms in the control tank water should exceed[3]:

- 10 viable organisms per cubic meter greater than or equal to 50 micrometers in minimum dimension, and
- 10 viable organisms per millilitre less than 50 micrometers in minimum dimension and greater than or equal to 10 micrometers in minimum dimension
- no limits for bacteriae.

[1] G8, Annex, Part 2, paragraph 2.2.2.9
[2] G8, Annex, Part 2, paragraph 2.2.2.5
[3] G8, Annex, Part 2, paragraph 2.2.2.5

4. Test cycles

The amount of ballast water pumped onboard in the test cycle should be consistent with the normal ballast operations of the ship and the ballast water management system should be operated at the treatment rated capacity for which it is intended to be approved.

A shipboard test cycle includes:
- the uptake of ballast water;
- the storage of ballast water onboard, and
- the discharge of ballast water from the ship.

4.1. Number of test cycles

Three consecutive valid test cycles which are to span a trial period of not less than six months[4] need to be performed. Any invalid test cycle does not affect the consecutive sequence[5].

The control and treatment test cycles may be run simultaneously or sequentially at each sampling event.

4.2. Validity of test cycles

A treatment test cycle should be deemed successful (= valid) if:
- uptake water, both the control tank and ballast water to be treated, contains viable organism concentration exceeding 10 times the maximum permitted values in Regulation D-2.1 and control tank viable organism concentrations exceed the values of Regulation D-2.1 on discharge[6].
- the average density of organisms greater than or equal to 50 micrometers in minimum dimension in the replicate samples of the treated water upon discharge from the ship is less than 10 viable organisms per cubic metre;
- the average density of organisms less than 50 micrometers and greater than or equal to 10 micrometers in minimum dimension in the replicate samples of the treated water upon discharge from the ship is less than 10 viable organisms per millilitre;
    - the average density of *Vibrio cholerae* (serotypes O1 and O139) is less than 1 cfu per 100 millilitres, or less than 1 cfu per 1 gramme (wet weight) zooplankton samples upon discharge from the ship;
    - the average density of *Escherichia coli* in the replicate samples is less than 250 cfu per 100 millilitres upon discharge from the ship; and
    - the average density of intestinal Enterococci in the replicate samples is less than 100 cfu per 100 millilitres upon discharge from the ship.

If in any test cycle the average discharge results from the control water is a concentration less than or equal to 10 times the values in Regulation D-2.1, the test cycle is invalid.

[4] G8, Annex, Part 2, paragraph 2.2.2.7
[5] G8, Annex, Part 2, paragraph 2.2.2.8
[6] G8, Annex, Part 2, paragraph 2.2.2.5

5. Sampling points

Sampling ballast *via* manholes and sounding or air-pipes is not used because these do not lead to representative sampling. Such sampling may underestimate the densities of organisms present. Consequently, samples should be taken at a sampling point along the ship's ballast water line as this provides direct access to the water.

Facilities or arrangements for sampling must be provided in the intake and discharge ballast water pipe of both the treated and control line to ensure that representative samples of treated and control water can be taken with as little adverse effects as possible on the organisms.

Sampling points should be installed into the ships ballast water piping system in a straight (at best horizontal) pipe section:

(a) prior the treatment system to evaluate whether untreated water samples attain the minimum density for organisms during intake, and

(b) as close as possible to the ballast water discharge point where the water exits the vessel to evaluate the treatment system performance, i.e. to proof whether or not the performance standard is met[7].

Sampling points for both the control and treated water tests should be identical in design. Guideline G2 gives some guidance how a sampling point may be designed:

> *In order to undertake an accurate measurement on the organism concentration in the ballast water, it is recommended to install an "isokinetic" sampling facility. Isokinetic sampling is intended for the sampling of water mixtures with secondary immiscible phases (i.e. sand or oil) in which there are substantial density differentials. In such conditions, convergence and divergence from sampling ports is of significant concern. Since most organisms are relatively neutrally buoyant, true isokinetic sampling is unnecessary. However, the mathematics related to isokinetic sampling are deemed to be useful as a basis for describing and specifying sampling geometries. Isokinetic sampling is necessary to ensure that a sample contains the same proportions of the various flowing constituents as the flow stream being sampled. During isokinetic sampling the sampling device does not alter the profile or velocity of the flowing stream at the moment or point at which the sample is separated from the main flow stream. Under isokinetic conditions, the velocities of both the sample and the main flow are equal at the point at which the sample is separated from the main flow. To achieve isokinetic sampling conditions, a sampler is designed to separate a subsection of the total flow-stream in a manner that does not encourage or discourage water entry other than that which is otherwise in the cross-section of the sampler opening. In other words, flow streams in the main flow of the pipe should not diverge or converge as they approach the opening of the sampler.*

[7] Regulation D-2 reads "… ships shall not discharge…", i.e. the closer the sampling is located to the ships discharge point the better.

6. On-board sampling scenario

The following text describes the sampling scenario and sample analysis for onboard tests of ballast water treatment systems according to the IMO Guideline G8. The guideline states that the samples should be analysed as soon as possible after sampling was completed, and not later than within 6 hours after sampling.

Scientific studies have shown that organisms are not homogenously distributed inside ballast water tanks, but that they concentrate in certain water layers or tank areas. The location of the organism rich layers cannot be predicted as this depends on e.g. the holding time in the tank and ship movement influenced by weather conditions. Consequently, when missing the organism rich or poor water layers inside the tanks false positive results may be generated (i.e., BWM Convention compliant) (Gollasch & David 2010 a-c).

The IMO Guideline G8 permits different sampling approaches which is in-line with the nature of this instrument, i.e. being a guidance document.

One way of interpreting G8 is that for all uptake samples 1 sample in beginning, middle and end will be taken resulting in 3 x 1000 L samples for both uptake control and uptake treated, totals to 6 samples. Discharge samplings would result in 3 x 1000 L for control and 3 x 3 x 1000 L for treated water = 12 samples.

Working onboard is different than on land. Onboard logistics rarely permit to take so many samples. In addition to these logistical constraints, on discharge one biologist cannot work out 12 samples per organism category in Regulation D-2 in the required time frame of 6 hours in the same level of detail (sub-sample volume to analyse). Considering to bring another biologist to help is an option, but on most vessels the cabin and life boat capacity is limited that this cannot be done.

Further, there is believe that the sampling approach to take a sample in beginning, middle and end is less accurate (but see further below). Imagine the pumping time to fill and discharge the tank takes 60 minutes. This may result that the beginning sample to be taken from minute 1 to 10, the middle sample from min 25 to 35 and the end sample from min 50 to 60.

Consequently you sample approximately during half the pumping time. Studies have shown that there is organism patchiness in the ocean and when ballast water is discharged (Gollasch & David 2010 a, b). Sampling only half the time may result in missing organism rich layers of water, which is especially to consider during the discharge of the treated water. Imagine a tank is divided in 10 vertical layers. When sampling in beginning, middle and end, you may sample layers 1-2, 4-5 as well as 9-10. Layers 3 and 6-8 will not be sampled at all. What if the organisms have accumulated in layer 7? These would have been missed from sampling which may result in a false positive result, i.e. no living organisms found because the organism rich layer was not sampled.

To solve this dilemma samples are taken over the entire time of the pumping event. When taking a sample over the entire time there is no need to take three times three replicates of the discharged treated water. In G8 it was aimed to avoid that you sample until the required water volume of 1000 L was reached and then stop, which may result in sampling only during 25% or less of the water from the tank being emptied. Noting organism patchiness in the tank G8 aims to make sure that you sample more than one fraction of the tank and this is why beginning, middle and end samplings were included.

However, and in contrast with all stated above, regarding the sampling over the entire time, a research study showed disadvantageous in the over entire time samples. When sampling the same body of water (by using a flow splitter) the samples taken in the beginning, middle and end had always a higher number of organisms above 50 micron in minimum dimension compared to the over entire time samples. For smaller organisms and bacteria such a clear trend could not be observed (Gollasch & David 2010 c). One conclusion was that the over entire time sample usually resulted in larger volumes of water sampled and therefore the organisms in the sample of the untreated ballast water are concentrated to much higher numbers per water volume compared to what they are exposed to in nature (crowding effect). Further, the over entire time samples take longer compared to the beginning, middle and end samples thereby exposing the collected organisms to a longer time during the sampling event. These two situations may increase the organism mortality. It should be noted that these results are based upon studies with untreated ballast water, i.e. with high numbers of organisms. Consequently the crowding and time effects were likely much higher in the above mentioned studies compared to sampling treated water because in treated water the organism concentration will be orders of magnitude lower.

In conclusion, the above described sampling scenario with samples taken over the entire pumping time may have impacts on the survival of larger organisms in organism rich water (uptake control and before the treatment system, and discharge of control water). For the treated water at discharge, where the organism concentration is lower than in nature as a result of the treatment process the crowding effect should be neglectable. Consequently, the over the entire time sampling approach seems to be the best compromise for onboard work on commercial vessels due to logistical and time constraints. It should be noted that this approach was accepted by the French, Greek, German, South African and United Kingdom administrations.

6.1. Samplings at ballast water uptake

The sampling for all organism groups should be undertaken in parallel, i.e. samples for all three organisms groups as outlined in Regulation D-2 should be taken at the same time. The setup is shown in Figure 1.

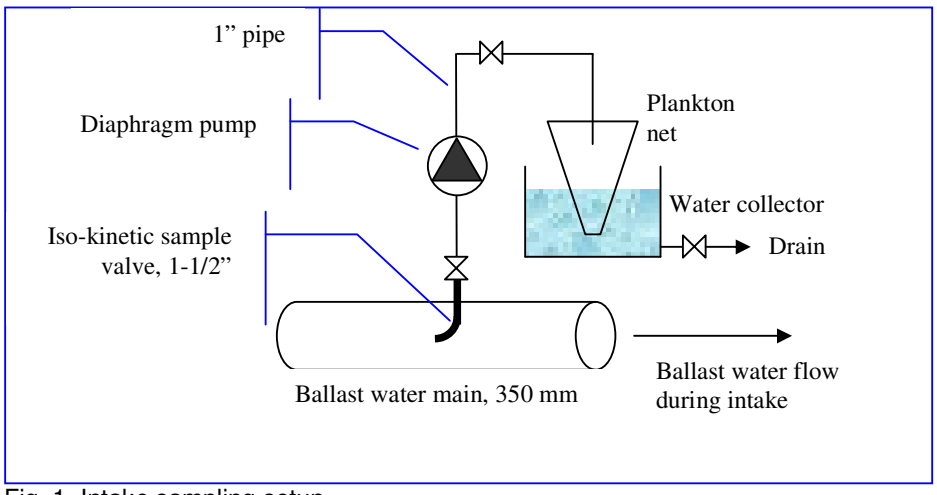

Fig. 1. Intake sampling setup.

6.1.1. Abiotic water conditions

In addition to biological samplings, the source water for the test cycles shall be characterized by measurement of salinity, temperature, particulate organic carbon and total suspended solids. In minimum this should be done during ballast water uptake, but may also be considered during discharge.

6.1.2. Organisms above 50 micron in minimum dimension

As the control and treated tank cannot be filled simultaneously one sampling event is needed for each tank (control and treated). For each sample at least 1 tonne of water needs to be sampled which will be done over the entire uptake time (= at least 1 tonne of water).

After sampling, the water sampled may be pumped back into the ships´ ballast water discharge line, dumped in the bilge water system or discarded otherwise.

Sampling such large volumes of water is a challenge. To be enabled to sample such large volumes of water the German vendor HydroBios developed a sampling kit which consists of filtering bag and removable cod-ends with a mesh of 50 micron in diagonal dimension. An integrated flow meter allows for exact measurements of the filtered water volume. Previous onboard tests have proven that this sampling kit efficiently sampled up to 2,500 litres of water in less than 30 minutes (Fig. 2). In case of extremely high organisms and/or sediment loads in the water several cod-ends may be used. Multiple sets of this tool will be used to enable replicate samplings of the treated water at discharge.

Fig. 2. Newly designed ballast water sampling tool. Note water collection buckets (centre) below the nets to avoid water spillage in the ships´ bilge water system. The removable cod-end is shown on the right photo.

6.1.3. Organisms below 50 micron and above 10 micron in minimum dimension

As the control and treated tank cannot be filled simultaneously one sampling event is needed for each tank (control and treated). For each sample at least 1 litre of water needs to be sampled which will be done over the entire uptake time (= at least 1 litre of water).

Samples will be collected with a measuring cup multiple times (every few minutes) as a subsample of the water sampled for organisms above 50 micron in minimum dimension, i.e. at the flow meter outlet into the HydroBios sampling nets.

6.1.4. Bacteriae

As the control and treated tank cannot be filled simultaneously one sampling event is needed for each tank (control and treated). For each sample at least 500 millilitres of water needs to be sampled which will be done over the entire uptake time (= at least 500 millitres of water).

Samples will be collected with a measuring cup multiple times (every few minutes) as a subsample of the water sampled for organisms above 50 micron in minimum dimension, i.e. at the flow meter outlet into the HydroBios sampling nets.

6.2. Samplings at ballast water discharge

The control and treated tank are emptied consecutively which results in two sampling events during discharge. It is recommended to discharge and sample the treated tank first to avoid contamination with individuals from the likely more organism rich control tank. The sampling setup for the treated water at discharge is shown in Figure 3.

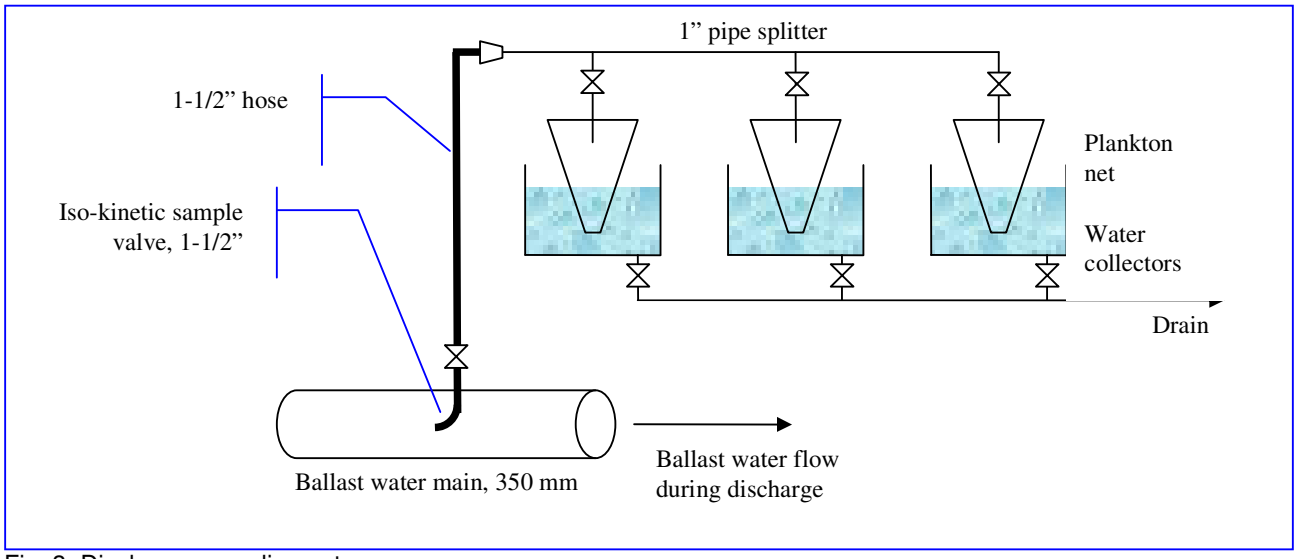

Fig. 3. Discharge sampling setup.

6.2.1. Organisms above 50 micron in minimum dimension

For the control tank organisms above 50 micron in minimum dimension at least 1 tonne of water needs to be sampled over the entire time (= at least 1 tonne of water).

Samples from the treated tank should be taken in triplicate, i.e. for organisms above 50 micron in minimum dimension at least 3 times 1 tonne of water needs to be sampled over the entire discharge time (= at least 3 tonnes of water).

6.2.2. Organisms below 50 micron and above 10 micron in minimum dimension

For the control tank organisms below 50 and above 10 micron in minimum dimension at least 1 litre of water needs to be sampled over the entire discharge time (= at least 1 litre of water).

Samples from the treated tank should be taken in triplicate, i.e. for organisms below 50 and above 10 micron in minimum dimension at least 3 times 1 litre of water needs to be sampled over the entire discharge time (= at least 3 litres of water).

Samples will be collected with a measuring cup multiple times (every few minutes) as a subsample of the water sampled for organisms above 50 micron in minimum dimension, i.e. at the flow meter outlet into the HydroBios sampling nets.

6.2.3. Bacteriae

For the control tank bacteriae at least 500 millilitres of water need to be sampled over the entire discharge time (= at least 500 millilitres of water).

Samples from the treated tank should be taken in triplicate, i.e. for bacteriae at least 3 times 500 millilitres of water need to be sampled over the entire discharge time (= at least 1500 millilitres of water).

Samples will be collected with a measuring cup multiple times (every few minutes) as a subsample of the water sampled for organisms above 50 micron in minimum dimension, i.e. at the flow meter outlet into the HydroBios sampling nets.

6.3. Sample number summary

The following table provides a summary of the number of samples and their volumes to be taken during on board tests for one test cycle.

Table 1 Number of samples and their volumes for one test cycle according to G8. In case the treated and control tanks can be filled in parallel one sampling event is sufficient during uptake. It was further assumed that the sampling time stretches over the entire uptake or discharge time of the ballast tanks (see also Annex 1). The numbers referring to paragraphs refer to IMO Guideline G8.

Sample purpose	**Treated tank**		**Control tank**	
	uptake (para 2.2.2.5 and 2.2.2.6)	discharge (para 2.2.2.6.2 and 2.2.2.6)	uptake (para 2.2.2.6.1 and 2.2.2.6.3)	discharge (para 2.2.2.6.1 and 2.2.2.6.3)
Environmental parameters[8]	1 sample	1 sample (recommended, but not required in G8)	1 sample	1 sample (recommended, but not required in G8)
>50 µm[9]	1 x >1000 L (recommended, but not required in G8)	3 x >1000 L	1 x >1000 L	1 x >1000 L
<50 to >10 µm	1 x >1 L (recommended, but not required in G8)	3 x >1 L	1 x >1 L	1 x >1 L
Bacteriae	1 x >500 ml (recommended, but not required in G8)	3 x >500 ml	1 x >500 ml	1 x >500 ml (recommended, but not required in G8)

6.4. Sample processing

The samples should be processed as soon as possible after the sampling event and all attempts should be undertaken to complete the sample analysis of all samples within 6 hours after sampling. The 6 hours time limit refers to relatively short generation times of certain taxa and possible mortality in storage prior to analysis may have an impact even after a few hours.

For all biological samples the likely organism-poor treated water samples should be processed first to avoid organism "contamination" with the likely organism-rich control samples.

In case the water temperature of the sampled water is below room temperature all sample buckets and the filled 1 L bottles for bacteria analysis should be placed in an environment with similar temperature to avoid heating up of the sample water which may negatively impact organism survival. To do this, samples may need to be stored in a fridge of cooling room until analysis as practicable.

[8] Temperature, salinity, total suspended solids and particulate organic carbon, see G8, Annex, Part 2, paragraph 2.2.2.9

[9] There is an inconsistency and unclear wording in G8 regarding the uptake sample of the treated line. G8, Annex, Part 2, paragraph 2.2.2.5 *Valid tests are indicated by uptake water, for both the control tank and ballast water to be treated, with viable organism concentration…* but 2.2.2.6 *Sampling regime* sets only requirements for the discharge of treated water. To be on the save side samples during uptake of the treated water are therefore recommended.

6.4.1. Abiotic conditions

The source water for test cycles shall be characterized by measurement of salinity, temperature, particulate organic carbon and total suspended solids[10]. All abiotic parameters should be measured in all samples (but see Table 1).

For salinity measurements a calibrated electronic measurement device will be used. A sample volume of less than 1 litre is considered sufficient. This measurement will either be done at the sampling point or later in the cabin.

In parallel to salinity measurements, the water temperature should be measured with an electronic measurement. This is to be done during the sampling event at the sampling point.

The particulate organic carbon (POC) and total suspended solids (TSS) analysis will be carried out on land after the voyage by NIOZ, Texel, the Netherlands. For this analysis fibre filters (e.g. Whitman GF/C) will be dried overnight at 500 °C, weighted and put in numbered and sealed aluminium foil or Petri discs. The filters are provided by NIOZ. Onboard, water is filtered and thereafter the filter is rinsed three times with 10 ml fresh water (preferably distilled water) and put back in the original Petri disc. The filters are thereafter frozen at ca. – 20 °C (in the ships deep freezer) and should at best be kept frozen until analysis. Preferably 1 litre of water should be vacuum filtered for TSS.

For sample shipment the sample transfer protocol will be followed (Annex 3).

6.4.2. Organisms minimum dimension measurement

For organisms above 10 micron the minimum dimension measurement should be based upon an investigation of the organism "body", thereby ignoring sizes of thin spines, antenna etc (Fig. 4). In e.g. flat worms or diatoms the minimum dimension should be the smallest part of their "body", i.e. the dimension between the body surfaces when looked at the individual from the side.

In ball shaped organisms the minimum dimension should be the spherical diameter.

In conclusion, the smallest visible axis should be chosen and the widest point on this axis be measured. If organisms are near the 50 um minimum dimension, efforts should be made to measure the third (less visible) dimension.

This approach is in-line with the views expressed at the relevant IMO discussions and with the findings of a workshop held in the framework of the Interreg IVB Project Ballast Water Opportunity in October 2010. This workshop was attended by several test facilities of ballast water treatment systems including representatives from Danish Hydraulic Institute, Hørsholm (Denmark), GoConsult, Hamburg (Germany), Golden Bear Facility, Vallejo (USA), Great Ships Initiative, Superior, Wisconsin (USA), IMARES Wageningen UR, Den Helder (the Netherlands), Royal Netherlands Institute for Sea Research, Texel (the Netherlands) and Maritime Environmental Resource Center, Solomons, Maryland (USA).

[10] G8, Annex, Part 2, paragraph 2.2.2.9

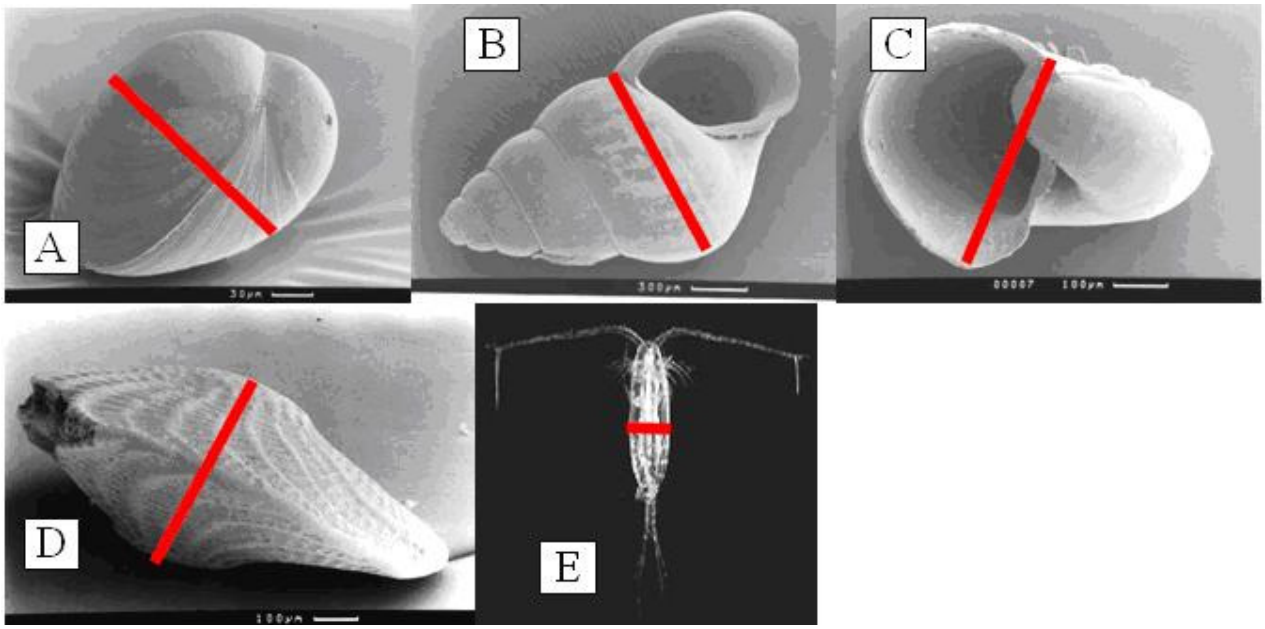

Fig. 4 Minimum dimension measurements (red line) for selected organism types: A = mussel larvae, B and C = gastropod larvae, D = Foraminifera (phytoplankton), E = copepod. All organisms shown here are well above 50 micron and organisms shown in A to D were sampled from ballast water during the German shipping study (Photos A - D: Stephan Gollasch, E: www.wikipedia.org).

6.4.3. Organism counting

6.4.3.1. Organisms above 50 micron in minimum dimension

Samples will be analysed onboard directly after sampling. A stereo-microscope will be used for counting the larger organisms in the samples (Fig. 5). Illumination is provided from the top or bottom.

Fig. 5 A stereo-microscope in use for onboard analysis of organisms above 50 micron. A Bogorov counting chamber is shown on the white tray in the foreground (Photo: Stephan Gollasch).

Organisms greater than or equal to 50 micrometers in minimum dimension should be counted under magnifications of at least 10 x. For a size measurements a piece of the filtering mesh (50 micron in diagonal dimension) will be put under the stereomicroscope. This transparent mesh is used as a scale and all living organisms above 50 micron in minimum dimension will be counted. The organism numbers will be recorded according to broad taxonomic groups, such as copepods, decapods, polychaetes, bivalves, gastropods, phytoplankton etc. (see Annex 1).

In case samples need to be concentrated a sieve no bigger than 30 micron should be used, except in waters with a very high load of sediments. In those cases using the smaller meshsize will result in very long filtration times which may negatively impact organism survival. Therefore, for concentrating waters with a very high sediment load it is recommended to use a mesh of 50 micrometers in minimum dimension.

The concentrated sample should not be less than 50 millilitres in total volume to avoid negative effects of the densely concentrated organisms. The samples should be analysed as soon as possible after concentration.

In organism rich samples (e.g. uptake water, discharge sample of the control tank) the sample may be divided into sub-samples. This may be undertaken by proper mixing of the sample (stir well until organisms are equally distributed in the sample) and extracting when practicable approximately 10 millilitres with a pipette[11] for analysis. Organism poor samples (e.g. the samples taken from the treated tank upon discharge) should be analysed completely when practicable.

When using Petri discs counting may not be accurate as the ship movement induces water movements in the Petri disc. As a result organisms may be counted twice and some may be missed out from counting. To avoid this, a Bogorov counting chamber may be used. During minimal ship movements, this chamber proved to be efficient during onboard trials. However, with increasing ship movements the Bogorov chamber loses its advantage. HydroBios therefore designed three new counting chambers which may be used with increasing ship movements. Using these gives a much greater accuracy as here the size of the water canal width corresponds to the stereomicroscope observation field of view thereby reducing the risk to overlook organisms (Fig. 6). Count the organisms as soon as possible after the counting chambers were filled with the concentrated sample water. Longer waiting times may negatively impact organism survival in the counting chamber.

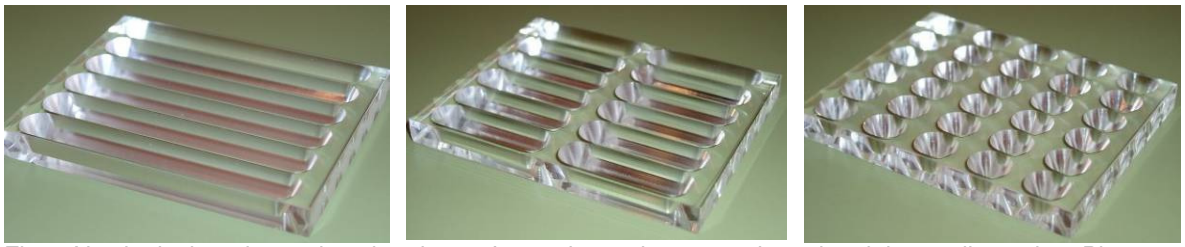

Fig. 6 Newly designed counting chambers of organisms above 50 micron in minimum dimension. Photos courtesy of www.hydrobios.de.

[11] A new pipette will be used for each sample to avoid organism contamination.

6.4.3.2. Organisms below 50 and above 10 micron in minimum dimension

Zooplankton organisms in the less than 50 and greater than 10 micrometer in minimum dimension will be analysed onboard directly after sampling with a similar method as for the zooplankton organisms above 50 micron, but using a magnification of at least 20 x.

For phytoplankton an unconcentrated 1:1 sample will be taken for later analysis of cells with the flow camera on land. Optional the phytoplankton viability may be analysed by using a Pulse-Amplitude Modulated (PAM) fluorometer on board. The PAM will deliver viability data and the flow camera analysis on land will count cells. In combination PAM and flow camera data will then be used to identify the number of viable organisms.

For analysis of smaller phytoplankton organisms a Coulter Counter fluorometer or similar tool will be used in the land-based laboratory (NIOZ). This tool will automatically count objects per size class and is also able to assess organism viability for phytoplankton with the use of FDA stains together with epifluorescence microscopy or flow cytometry. In general these tools are applied to distinguish living phytoplankton from other detrital material and zooplankton based on the presence of auto chlorophyll fluorescence of phytoplankton. Three replicate samples of 3 mL each will be taken from both control and treated water. They will be pipetted in an ultra clean sampling tube and put in the carousel of a bench top flow cytometer (Beckman Coulter XL-MCL). This flow cytometer is certified for medical laboratory use and as such maintained by the manufacturer in their service scheme. All procedure and handling are conducted according the standard procedures as described in Shapiro (2003). As a light source a 15 mW Argon laser is used (488 nm excitation wavelength). Forward and side scatter is detected of each particle as well as the fluorescent emission in the yellow/green (525 ± 20 BP filter), orange (575 ± 20 nm) and red wavelength band (>645 nm). Samples will be counted using a standard protocol covering the particles in the size range of ca. 2 to 50 µm. Total analysis time will be equal to an exact sampling volume of 1 ml. Of all particles present in 1 ml of sample, cell size and presence or absence of chlorophyll a will be measured. Absolute numbers, cell sizes and chlorophyll *a* content of the particles will be analyzed using the software package FCS Express V3 (DeNovo version 3). Cell sizes will be estimated relative to 10 µm standard fluorescent beads (Flow-Check Fluorospheres, Beckman Coulter #660539). Phytoplankton will be distinguished from other particles based on the presence of chlorophyll *a*, resulting a bright red fluorescent signal (emission > 645 nm) (Peter Paul Stehouwer, NIOZ, pers. comm.).

In addition one set of samples will be preserved using Lugol Solution and another set be kept alive in a fridge. Both sets will later be analysed in a land-based laboratory (at NIOZ, Texel, The Netherlands). In case samples will be sent to a land-based laboratory the sample transfer protocol will be followed (Annex 3) as a chain-of-custody procedure.

Colony forming species - A question arose in which size category a colony falls when the single cell is below 50 micrometres but the colony is above 50 micrometres. A team of experts, i.e. the ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors, believes that in those cases the individual specimen size should be measured. This group finding is based upon the D-2 standard as it refers to organisms and not to colonies. Further, viability assessments should address the smallest unit able to reproduce which is the individual and not the colony. Based on this conclusion the size of the individuals should be measured and not the colony.

6.4.3.3. Bacteriae

Except for *Vibrio cholerae* bacteria will be cultured on board using selective media (see below). Cholerae bacteria will be analysed in a certified laboratory on land (IBEN, Bremerhaven, Germany).

6.5. Viability assessment

6.5.1. Organisms above 50 micron in minimum dimension

The organism viability may be assessed by visual inspection, i.e. all individuals should be inspected for movement or organ activity. The life/dead judgement should be undertaken by exposure of the organisms to light (under the stereomicroscope) and in addition non-moving organisms should be poked with a needle to initiate movement. Intact and moving organisms should be considered as living, organ activity of intact organisms gives an additional indication of viability. This assessment should be undertaken on board and completed for all samples no later than 6 hours after sampling.

6.5.2. Organisms below 50 micron and above 10 micron in minimum dimension

Stains together with epifluorescence microscopy or flow cytometry will be used to proof viability of phytoplankton organisms. In general these tools are applied to distinguish living phytoplankton from other detrital material and zooplankton based on the presence of auto chlorophyll fluorescence of phytoplankton.

To proof viability of phytoplankton the Pulse-Amplitude Modulated (PAM) fluorometer method may be used on board whenever possible. Should the PAM not be available for on board use, NIOZ will measure the phytoplankton viability in addition also by using a PAM.

PAM fluorometry
The photochemical efficiency of photosystem II (an indicator of the 'health' condition of the cell) of phytoplankton can be assessed using the Pulse-Amplitude Modulated fluorometer (Schreiber et al 1993, Fig. 7). This simple parameter gives a qualitative indication of the photosynthetic activity of the phytoplankton community.

Fig. 7 PAM fluorometry; a fast method to determine (bulk) phytoplankton biomass and the physiological condition of the photosynthetic apparatus of the cells.

The PAM-fluorometer will be calibrated with water filtered through a 0,45 µm pore size filter (Fig. 8) and the result taken as the "zero sample". As a second step sample water will be filtered through a sieve with a meshsize of 50 micron in minimum dimension and the water filtered through this net will be filtered again through a sieve with a meshsize of 10 micron in minimum dimension. This results in a concentration of cells smaller than 50 micron and larger than 10 micron in minimum dimension. After calibration 3 ml of the filtered sample water are filled into a glass cuvette and are analysed using the PAM-fluorometer in an automated measurement. As a result the viability of the phytoplankton cells is measured. The Lugol preserved samples will later be analysed in the NIOZ laboratories for the number of cells in the sample. Consequently, the on board viability test in combination with the land-based cell counts provide the necessary results of viable cells per water volume. The on board measurement should be undertaken and completed for all samples no later than 6 hours after sampling.

As a back-up one set of unconcentrated samples will be preserved with Lugol solution in case later analysis on land becomes necessary. If needed samples should be placed in a settlement chamber overnight. Organisms should be counted by using magnifications of at least 100 x of an inverted microscope. Stains may also be used to assess organism viability. In phytoplankton species an inspection of the photopigments may reveal additional information to assess viability.

The viability of zooplankton organisms in this size class will be analysed on board with the same method as for the zooplankton organisms above 50 micron. This assessment will be completed for all samples no later than 6 hours after sampling.

6.5.3. Bacteriae

Selective growth media, an immunoassay or quantitative PCR (Polymerase chain reaction) should be used for the three categories of organisms mentioned in G8 i.e. toxigenic *Vibrio cholerae* (serotypes O1 and O139), *Escherichia coli* and intestinal *Enterococci.* Where appropriate a dilution series will be applied to ensure proper counting of bacteria. By doing so bacteria counting is enabled even in the unlikely case of high concentrations. The different dilution cultures will be used to back-calculate the bacteria densities in the original sample. Where appropriate, for each bacterial sample at least two culture trials should be started in parallel.

To avoid contamination the desk and other working items are cleaned with Ethanol prior sample processing.

In case a dilution series is applied, i.e. for the Enterococci and *Eschericha coli* samples (see below), the Millipore filter stand does not need to be cleaned between water filtration events and further the same Millipore filter stand funnel may be used to filter all water volumes for all bacteria of one sample as the water source is identical.

Annex 11 shows the bacteria incubation times so that the operator onboard keeps record of the incubation not to miss the time window for analysis.

6.5.3.1. Vibrio cholerae (serotypes O1 and O139)

On board analysis is not permitted due to safety concerns. To analyse for the abundances of O1 and O139, samples will be stored for later analysis, e.g. by a monoclonal antibody tagged with

fluorescein isothiocyanate (FITC) immunoassay for specific disease causing strains. Alternatively quantitative PCR (Polymerase chain reaction) may be used, i.e. "copy" DNA, proof presence, multiply with dilution factor to back-calculate original numbers.

Samples will be sent to a land-based laboratory IBEN, Bremerhaven, Germany and the sample transfer protocol will be followed (Annex 3).

On board a Millipore filter set will be used to filter 100 ml of undiluted sample through a Whatman filter (0,45 µm pore size) (Fig. 8). Before doing so, the filter stand is cleaned with Ethanol and flame (Fig. 9).

Put filter in transport container, add a few millilitres of filtered water to avoid the filter to dry (Fig. 10) and keep in incubator (Fig. 11) at 37 °C for later analysis on land.

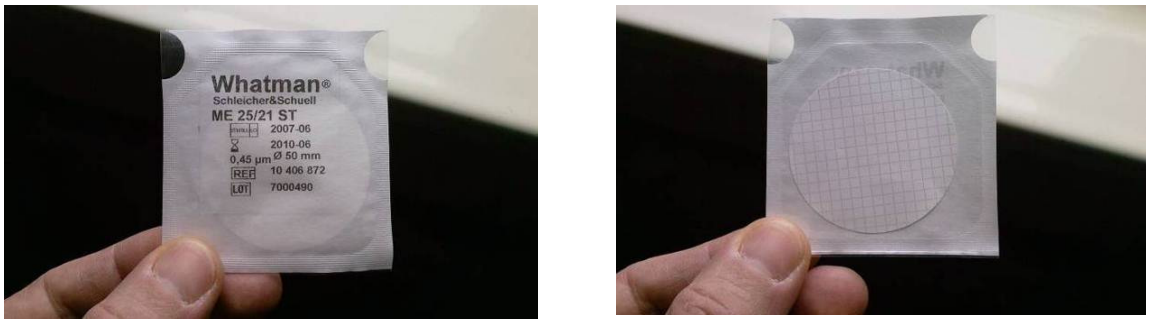

Fig. 8. Whatman filters (0,45 µm pore size) (Photo: Stephan Gollasch).


Fig. 9 Cleaning of Millipore stand (Photo: Stephan Gollasch).


Fig. 10 Whatman filter with *V. cholerae* sample (Photo: Stephan Gollasch).

6.5.3.2. Escherichia coli

It is recommended to follow the ISO standard 9308-1:2000 to analyse the samples for *E. coli* concentrations. A selective medium for this bacterium and the analysis is relatively easy, i.e. can be carried out on board. GoConsult is prepared to work according to ISO 9308-1:2000 and a bacteria culture chamber is available (Fig. 11).

Fig. 11. Incubator for Escherichia coli and Enterococci cultures (Photo Stephan Gollasch).

Use Millipore filter set to filter, when appropriate, 1 ml, 10 ml and 100 ml of undiluted sample through Whatman filter (0,45 µm pore size) (Fig. 8). Before doing so, clean filter stand with Ethanol and flame it (Fig. 9). Make sure that the filter plate is as dry as possible before transferring it to the Petri dish.

Moisture the Petri dish with selective medium (Dr. Möller & Schmelz Lactose-TTC-Tergitol NPS (Order No. 1092)) with 3.5 ml of aqua dest. Add Whatman filters to Petri disc with medium. Place dishes from one sampling event into small plastic bag, but separate control and treated water samples. Incubate 21 +/- 3 hours at 36 +/- 2 °C. Bacteria of the *Escherichia* Genus form flat yellow colonies with yellow halo visible from top and bottom view of Petri disc (Fig. 12). Lactose negative bacteriae are either inhibited or develop small reddish colonies. Another indication is the turbidity of the fluid inside the Petri dish. In case the fluid is clear it is not likely to indicate an E. coli presence and turbid fluids indicate the presence of E. coli.

However, this method was developed to check for lower concentrations of E. coli. For higher bacteria densities this method is not suitable and here ISO 9308-3 is recommended. However, ISO 9308-3 cannot be carried out on board ships as microtiter plates cannot be sealed which may result in water spillage due to heavy seas or when the samples need to be carried from the ship to the port for an extended culture period. A second method in accordance with ISO 9308-3 was developed by idexx (USA). This requires larger incubators to store the idexx culture plates and also additional gear, i.e. a sealer to close the culture plates. This additional gear complicates the logistics, especially as all material needs to be shipped by air to and from the vessel. Further, earlier studies have shown that the bacteria density in sea water is very low. Consequently, due to low bacteria concentrations in sea water and also logistical problem the suitability of ISO 9308-1:2000 is indicated. To ensure that even in exceptionally higher bacteria concentrations samples can be analysed, a dilution series will be applied.

Please note also that the application of ISO 9308-1:2000 for the on board analysis of E. coli in tests of ballast water treatment systems was previously accepted by administrations, e.g. the Bundesamt für Seeschifffahrt und Hydrographie, Hamburg, Germany, Det Norske Veritas, Oslo, Norway and South Africa.

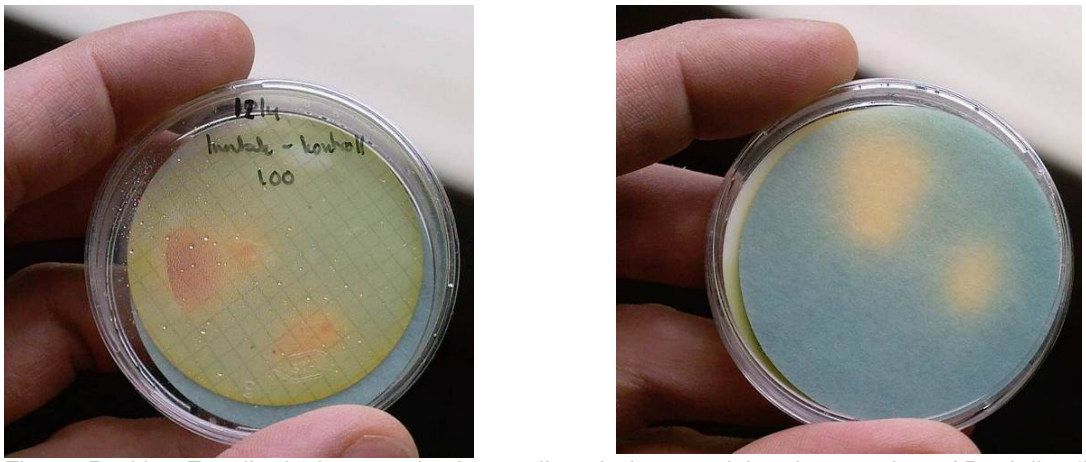

Fig 12. Positive *E. coli* colonies on selective medium. Left = top, right = bottom view of Petri disc (Photo: Stephan Gollasch).

In case yellow colonies with yellow halo visible from top and bottom view of Petri disc (Fig. 12) are found, take a photo of the Petri dishes from top and bottom. As a second step to proof positive *E. coli* colonies on the selective medium, transfer positive colonies to Tryptophane medium and incubate for 1 day at 44 °C. Positive *E. coli* are indicated by a colour change of the Tryptophane medium from yellow to red/purple when adding two drops of Kovacs solution: CH_3-$(CH_2)_3$-OH (Fig. 13).

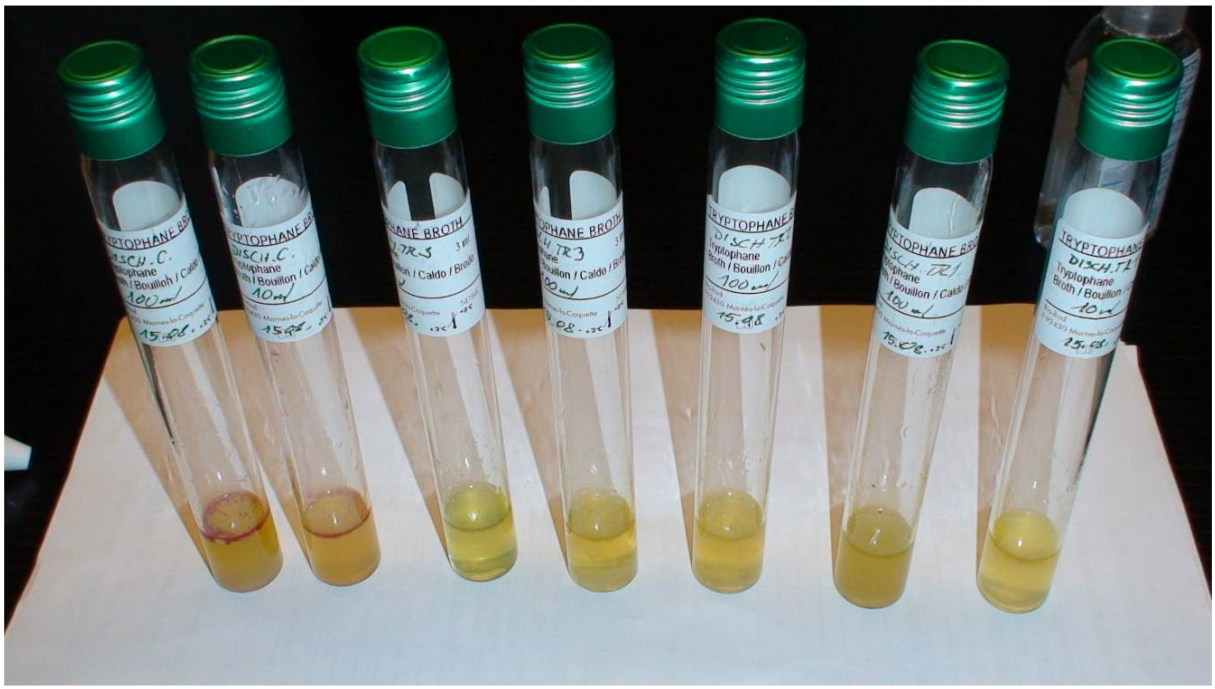

Fig. 13 Tryptophane medium incubated to proof *E. coli*. The two left test tubes show a positive result by a colour change from yellow to pink (Photo: Matej David).

6.5.3.3. Intestinal Enterococci

It is recommended to follow the ISO standard 7899-2:2000 to analyse the samples for *Enterococci* concentrations. A selective medium for this bacterium and the analysis is relatively easy, i.e. can be carried out on board. GoConsult is prepared to work according to ISO 7899-2:2000 and a bacteria culture chamber is available (Fig. 11).

Use Millipore filter set to filter, when appropriate, 1 ml, 10 ml and 100 ml of undiluted sample through Whatman filter (0,45 µm pore size) (Fig. 8). Before doing so, clean filter stand with Ethanol and flame it (Fig. 9).

Moisture the Petri disc with selective medium (Dr. Möller & Schmelz *Enterococcus* selective NPS medium (Order No. 1091)) with 3.5 ml of aqua dest. Add Whatman filters to Petri dish with medium. Place dishes from one sampling event into small plastic bag, but separate control and treated water samples. In case the samples need to be taken on land before the incubation time is completed, secure Petri dish lid with tape before placing the dishes into the plastic bag. At best incubate 48 hours at 36 +/- 2 °C. *Enterococci* form small, round and elevated pink or dark red colonies and the colonies are throughout in the same colour or with a darker spot in the center.

In case such colonies are visible, take a photo of all Petri dishes.

7. Cleaning

7.1. Treatment system

The ballast water treatment system needs to be clean, i.e. shall not contain living organisms prior each test run. This requires proper flushing and e.g. filter cleaning where appropriate.

It should be noted that organism can survive in the ballast water pipe work of the vessel. When working on ballast water discharge samples it is therefore recommend to work from organism poor to organism rich samples to avoid organism "contamination" between such samples. As a consequence the treated tank should be emptied first and before the sampling starts flushing of the ships ballast water pipe should be undertaken to ensure that only treated water is sampled and not untreated water from previous ballast water operations after the treated tank was filled.

7.2. Sampling equipment

After each sampling trial is completed, all sampling equipment and sample processing devices should be cleaned by using freshwater. All gear should also be inspected before any new samples are taken to prevent contamination with organisms from the previous sampling.

In addition, after completion of the sampling event, the HydroBios nets should be put in a big bucket and the bucket should be filled with water and detergent until the nets are completely covered. Let the nets sit in this cleaning solution overnight and rinse them well with freshwater. This cleaning exercise may be undertaken on land after the voyage.

7.3. Ballast tank

It is also recommended that the selected ballast tanks should be cleaned before commencing a test cycle. This may be achieved by multiple tank washings using e.g. the empty/refill method. Otherwise, organisms remaining in the tank from previous ballast water operations (including earlier treatment system tests) could interfere with the results.

8. Reporting and communication

A variety of data will be acquired and recorded electronically onboard by using laptop computers and manually (e.g., organism counts) by the GoConsult team. After each test cycle a test cycle report will be prepared by the sampling crew leader (see Annex 4) and an electronic copy of the data will be stored permanently along with all other study data. The second GoConsult expert is also provided with a copy of all electronical data prior leaving the vessel as a back-up.

In addition all electronically recorded data will be copied to a memory stick which is to be stored in a different location than the laptop computer to avoid losing data should either the memory stick or laptop computer get lost.

All sampling crew members planning to send samples to land-based laboratories follow the chain-of–custody protocol as shown in the Sample Transfer Document (see Annex 3).

The appointed sampling team leader will be responsible to record the data during sampling events at the sampling point. The essentials to document during uptake and discharge samplings at the sampling point are given in table format in Annex 5. These data should be transferred to the sample analysis sheet for organisms above 50 micron in minimum dimension (Annex 1) and also to complete the Test Cycle Report (Annex 4).

Items needed at the sampling point for uptake and discharge sampling events are stated in Annex 7.

Steps to set up the sampling gear are outlined in Annex 9.

For sample labelling use the labelling suggestions given in Annex 8.

The appointed sampling team leader will also be responsible to prepare test cycle result reports for each individual test cycle undertaken. These reports may follow the structure as described in Annex 4.

To ensure that all samples for later land-based analysis are taken from the vessel Annex 10 may be hang to the cabin wall as a reminder.

As it is sometimes essential to arrange a meeting with all sampling team members at short notice a "**sampling team locator"** may be printed and hang on the cabin door of each sampling team member (Annex 6).

9. Heavy seas

Safety first! The sampling team should always use "one hand for themselves and the other for the vessel", i.e. when carrying material to the sampling point or back to the cabin, carry only one bucket or bag at a time and hold to the hand rail, especially on stairs.

In case of heavy seas all fragile items must be placed on the floor, including

- Incubators,
- stereo microscope,
- flow meters, and
- computer.

When using the material above, even at calm seas always use an anti-slip matt on the working desk.

To avoid sample jars (concentrated zoo- and phytoplankton samples) and preservatives to flip over in heavier sea conditions, put these jars in empty coffee mugs from the ship with some Kleenex. Mugs less likely flip over as they have a better stand.

10. Calibration

The gear selected to undertake the sampling is light and robust at the same time to enable air travel without gear damage. All gear is checked prior packing and certain gear items are calibrated before packing. This in particular refers to the flow-meters and the salinity and temperature measuring instrument used. Further the incubators are checked for temperature stability and the gear for bacteria analysis is sterilised. The PAM instrument for phytoplankton viability is calibrated onboard. In a land-based process, the PAM performance is validated by a comparison of two identical PAMs in combination with traditional microscope phytoplankton counts. This is done annually by using living phytoplankton cultures.

10.1. Flow-meters

The flow meters are tested for accurate reading by pumping a known volume of water through the meter. In case adjustments are needed, which so far never was the case, the setting instructions of the manufacturer will be followed and the software adjusted accordingly.

10.2. Salinity and temperature measuring instrument

This WTW conductivity measuring instrument, which also measures the water temperature, is calibrated prior to each test voyages according the manufactures instructions. To do so the calibration standard test fluid for conductivity cells (0.01 mol/l KCl) is used according to ISO 7888.

10.3. Incubators

Prior the test voyages the incubators are run for a period of 2 days and the temperature stability is observed. In cases the temperature is fluctuating too much this incubator will be replaced or reset according to the manufacturer's instructions. Should the temperature drop e.g. during transport of

the incubators from the vessel to a hotel in cases where the bacteria culturing cannot be completed onboard, the incubation temperature should not drop less than 20 °C. To ensure proper incubator transportation a power converter is available to enable power supply during car driving.

10.4. Gear for bacteriae analysis

Prior the test voyages all gear needed for bacteriae analysis, including pipette tips, filter plates, funnels, media, inoculation loops and one time use gloves are packed in smaller quantities and be sterilised by using a Berchtold UV treatment chamber with 30 minutes exposure time (Fig. 14).

Fig. 14. External and inside view showing the Berchtold UV sterilization chamber in operation (Photo Stephan Gollasch).

10.5. Pulse-Amplitude Modulated fluorometer (PAM)

The PAM-fluorometer used for the phytoplankton viability assessment will be calibrated with destilled water onboard and the result taken as the "zero sample".

10.6. Flow cytometry

The flow cytometer in the land-based laboratory at NIOZ, the Netherlands, will be calibrated volumetrically and with an internal standard. The sensitivity of the instrument will be determined using standard calibration beads (Flow-Check Fluorospheres, Beckman Coulter #660539). These beads have an exact size and fluorescent signal with a variation for the different parameters of < 2%

(HPCV[12]). These standards will be run every sampling day, however over 10 years of experience has shown that instrument stability is extremely high over periods of months. Since the samples of interest are loaded with a high content of detrital material total and group specific number of organisms will be corrected for blank counts.

11. QA checks

11.1. Representativeness

The issue of sample representativeness is discussed above. Please consult the relevant chapter.

11.2. Accuracy

Key items regarding accuracy include sample volume, organism counts and organism viability.

The reading of the sample volume flow meter is calibrated prior the tests thereby avoiding substantial reading errors. Accurate flow meter readings are relevant as the number of viable organisms is calculated per water volume sampled.

Organism counts and the viability assessment are addressed in the relevant chapters above.

11.3. Bias and comparability

All onboard sampling events are considered as unique. This in particular refers to the difference in organism numbers and diversity at intake, water temperature, salinity and sediment load. Even if multiple experiments will be undertaken at the same location the seasonal changes make all ballast water operations unique events. Therefore a comparison between test runs may only be done on a very limited scale.

11.4. Emergency plan

All gear essentially needed for the sample taking and processing is either very robust or can be repaired. Repair kits will be brought as appropriate. In addition one complete sampling kit is brought as replacements and spare material is available in cases a repair cannot be done onboard.

11.5. Check lists

The following check lists are attached to this report

- Equipment check list (see next chapter);
- Packing check list, items needed at the sampling point (Annex 7); and
- Sampling event details, documentation at the sampling point (Annex 5).

[12] CV stands for Coefficient of Variation, so the variation on these beads is very low. HPCV is the Half-Peak Coefficient of Variation, which is the CV as given by the flow cytometer.

12. Personnel and responsibilities

The sampling crew will consist of two GoConsult experts (see Annex 2). It is also expected that at least one member of the vendor will join the sampling team to run the treatment system which should only be done in close consultation with the vessel crew.

The work load during sampling and sample processing requires a sampling crew of at least two trained and experienced members, and it is recommended that each have their own specialities on sample processing. It is therefore recommended that the sampling crew consist of one (zoo-) plankton specialist, ideally with microbiological background. Each sampling crew member is responsible to bring the equipment needed for the onboard analysis.

One sampling team member may be replaced by a generalist, particularly if some of the samples will be processed later in a land-based laboratory.

All sampling crew members planning to send samples to land-based laboratories should follow the Sample Transfer Document (see Annex 3).

One of the GoConsultians will be appointed as sampling crew leader on each voyage. It may be of interest to note that the GoConsult onboard work was successfully audited by Det Norske Veritas (see Annex 12) and Loyds Register (see Annex 13).

During each test cycle the sampling crew will make sure that the sampling equipment is cleaned prior each sampling event, that the equipment is correctly installed, that the samples are taken from correct sampling points, that the necessary number and volume of samples are correctly taken and that sample handling and sample processing is correctly performed.

Responsibilities of the sampling crew leader

- Communication concerning the test between sampling crew (see sampling team locator, Annex 6) and the crew of the treatment system vendor and ship crew
- In close consultation with the crew of the treatment system vendor/ship crew the sampling crew leader will decide
    - when to start ballast water uptake
    - when to start the ballast water uptake sampling event
    - when to finish the sampling
    - when to start the ballast water discharge
    - when to start the ballast water sampling during discharge
    - when to finish the ballast water discharge sampling event
- Responsible for the set-up of sampling gear at the sampling point (Annex 9)
- Responsible for shipment of samples, if necessary (see Sample Transfer Protocol, Annex 3 and Sample Transport Reminder, Annex 10)
- Responsible for documentation (see Sampling event details, documentation at the sampling point, Annex 5; Sample labelling, Annex 8) and reporting of the results (see Test cycle report, Annex 4)

The leader of the sampling crew can transfer responsibilities or duties to other sampling crew members provided such transfers are properly documented.

Responsibility of specialist for organisms larger than 50 µm

- Prepare all equipment for sampling
- Prepare all equipment for sample processing

- Process and analyse all samples onboard
- Reporting of results to the sampling crew leader

Responsibility of specialist for organisms between 10- 50 µm

- Prepare all equipment for sampling
- Prepare all equipment for sample processing
- Process all samples
- Reporting of results to the sampling crew leader

Responsibility of microbiological specialist (bacteria)

- Prepare all equipment for bacteria sampling
- Process and analyse all samples onboard (*E. coli, Enterococci*)
- Prepare all bacteria samples for later land-based analysis (*Vibrio cholerae*)
- Reporting of results to the sampling crew leader

Responsibility of generalist

- Support the installation of sampling equipment
- Measure temperature
- Measure salinity
- Collect sample for TSS/POC
- Document other relevant data, such as start and end time of the sampling event, lat/lon data, water depth, distance to nearest land for the starting and endpoint of the sampling event (interview responsible officers at the wheel house)
- Optional photo documentation of the sampling event
- Reporting of data to the sampling crew leader

Reporting of test results

After each test cycle a test cycle report will be prepared by the sampling crew leader.

Equipment, check lists, for one test

Bacteria sampling and processing

- Four 1 L bottles to collect subsample from bucket filled at the sampling point for bacteriae analysis in cabin
- At least six sterile bottles to store *Vibrio cholerae* samples
- Styrofoam box and heating elements for Cholera transport
- Two incubators with adjustable temperature and thermometers
- *Enterococci* kit (Dr. Möller & Schmelz media)
- *E. coli* kit (Dr. Möller & Schmelz media, Tryptophane, Kovac solution)
- Inoculating loops
- Distilled water (at least two bottles, one for the uptake and another the discharge sample processing)
- Alcohol (at least 70 % Ethanol or similar) for disinfection of equipment
- Forceps
- Single use latex gloves
- Measuring cup for water volume measurement
- 1000-5000 ml Eppendorf pipette with tips, or 1 and 5 ml syringes
- Plastic bags to seal Petri discs
- Whatman or Millipore filters 0.45 µm
- Millipore stand, with hoses (and vacuum flask/pump)

- Filtration funnels, sterile
- Burner to flame Millipore stand (as replacement bring a conventional lighter or matches)
- Paper towels
- Material to label samples (see Annex 8)
- Copies of Sample Transfer Protocol for Cholera-samples

Smaller plankton (10-50 µm)

- At least 12 x >50 ml bottles (6 for one set of living samples and 6 x for Lugol preserved samples)
- Optional: Flow camera and relevant gear
- Lugol solution
- Pipette for adding Lugol to samples
- Material to label samples
- Transparent tape to secure label on samples
- Styrofoam box (also to be used for TSS/POC samples)
- Cooling containers with cooling units
- Robust tape to seal cooling containers
- Copies of Sample Transfer Protocol (Annex 5)

Larger plankton (larger than 50 µm) sampling and processing

- Four HydroBios plankton nets
- Four flow meters
- At least five removable cod-ends
- Replacement filtering mesh (one for each cod-end)
- At least 4 buckets, preferably with lids
- Wash bottles
- Small sieves with removable 30 µm plankton mesh (one for each sample)
- Zooplankton counting chambers (one canal per row, two canals per row and with holes)
- Forceps
- Needles for poking (life/dead judgement)
- Funnel
- Pipettes
- Small measuring cup (100 ml) for concentrated sample
- Stereo microscope with light
- Replacement light bulb
- Paper towels
- Copies of Test Cycle Report
- Copies of zooplankton analysis sheet

TSS, POC, temperature and salinity

- Filtering stand for filter (diameter 47 mm)
- Pre-weighted GF/C filters
- Wash bottle
- Forceps
- Aluminium foil or small plastic bags
- Volume measurer
- Filtration funnels, not sterile
- Thermometer
- Salinity meter

- Material to label samples
- Copies of Sample Transfer Protocol

Additional requirements for all samplings

- The sampling points must be correctly installed by the vendor
- All sampling points should be of identical design
- Hoses for water transfer from sampling point to position for sample collection
- Where appropriate, water tanks and a draining system at the position of the sample collection point
- Watch
- Paper and writing utensils including waterproof marker
- Laptop computer
- Memory stick
- Photo camera (to document bacteria results)

Work clothes

- Light boiler suit
- Hearing protection
- Suitable footwear, e.g. steel-cap (rubber) boots
- Gloves

Transfer of samples to any land based laboratory

In case of sample transfer, a transfer protocol must be issued (Annex 3). The document must state all parties being involved in the transfer, type of sample (water, filter etc.), number of samples, type of analysis (phytoplankton, TSS, Cholera etc.), date of transfer and signature of all party representatives who handled the sample. The sample recipient laboratory should copy the signed sample transfer document and make this available to the sampling crew. The transfer document should be kept in the archives of the sampling crew.

Please note also the sample transport reminder (Annex 10).

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Annex 1 Sampling event details and zooplankton analysis sheet.

Sampling day				Temperature °C		Salinity PSU		
Location				TSS filter number				
Sample type				Comment				
Sampling time	start end			Largest organism				
Sample volume	unconcentrated							
	concentrated							
	analysed	2 ml x						
	Copepoda	Rotatoria	Cladocera	Mollusc larvae	"Worms"			
living[13]								
total:								
(dead)								

Total number of living individuals in 1000 L:

Sampling day				Temperature °C		Salinity PSU		
Location				TSS filter number				
Sample type				Comment				
Sampling time	start end			Largest organism				
Sample volume	unconcentrated							
	concentrated							
	analysed	2 ml x						
	Copepoda	Rotatoria	Cladocera	Mollusc larvae	"Worms"			
living								
total:								
(dead)								

Total number of living individuals in 1000 L:

Date (note also the time the sample processing started and ended)

Signature

[13] Each line stands for one sub-sample analysis, i.e. one line per HydroBios counting plate analysed. Record organism numbers of all six plate channels separately.

Annex 2 Brief description of sampling team expertise

The GoConsult sampling teams have conducted more than 50 on board voyages to test the performance of ballast water treatment systems since 2006.

Stephan Gollasch, marine biologist (PhD), born in Hamburg, Germany in 1962. Current position: senior scientist at GoConsult, an independent consultancy company based in Hamburg, Germany (www.gollaschconsulting.de).
Since 2002 Gollasch is involved in various projects and programmes dealing with ballast water treatment systems, including land-based and ship-board efficacy tests. Other fields of expertise include risk assessment and the development of ballast water management scenarios. Since 1994 Gollasch is member of the German Delegation at IMO MEPC and represents Germany in the Ballast Water Working Group. From the beginning he is member of the ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors and was chairman of the group from 2000 to 2006. Gollasch sampled the ballast water of more than 200 vessels and he was involved in onboard tests of more than ten ballast water treatment systems.

Matej David (PhD) was born in Koper, Slovenia, in 1967. He finished navigation studies (Maritime Transport Engineer), sailed on merchant vessels and holds the License of Competence as Ship's Mate (officer). Since 2001 he is involved in ballast water researches. In 2002 he started as member of the Slovenian Delegation at IMO MEPC and also represents his country in the Ballast Water Working Group. In 2003 he was the leading scientists in a ballast water sampling study undertaken in the Port of Koper. He developed new ballast water sampling gear which was patented in 2003. In 2007 he got his doctoral degree in the field of maritime transport with a focus on ballast water management decision support aspects. In the Port of Hamburg, Germany, he was familiarized with the operation and sampling of a ballast water treatment system on a land-based test site (i.e. performance tests). He was involved in on board tests of more than ten ballast water treatment systems and on many of those ship voyages he was appointed as sampling team leader.

Jonathan **Dan Minchin**, marine biologist (PhD), born in Galway, Ireland in 1946. He has a broad knowledge of marine biota. He is a scientific diver and holds safety at sea certificates. He has specialised in aquatic alien species and has a background in aquaculture, fisheries and environmental management. He is a senior investigator on hull fouling and ballast water alien transmissions. He has been senior scientist on zooplankton research cruises and has been a member of the ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors representing Ireland. In the Port of Klaipeda, Lithuania he was familiarized with the operation and sampling for efficacy tests of a ballast water treatment system onboard a commercial vessel. Since he was involved in onboard tests of several ballast water treatment systems.

Annex 3 Sample Transfer Protocol

Sample Transfer Protocol

Project details: e.g. onboard test 1, dd.mm.yyyy to dd.mm.yyyy

The responsibility of these samples is transferred to the sample recipient by the signature of the recipient below.

Owner of sample(s) and results: Stephan Gollasch, GoConsult, Grosse Brunnenstr. 61, 22763 Hamburg, Germany, sgollasch@aol.com, Tel. +49 40 390 54 60

Sample recipient: details of analytical laboratory

Comment:
e.g. Please check for toxicogenic *Vibrio cholerae* (O1 and O139) and document density of colony forming unit (cfu) per 100 millilitres.
e.g. Please check for viable organisms below 50 and above 10 micron in minimum dimension and report densities of such individuals per water volume. Please measure volume of water in sampling jar.

Number of samples:

Sample labelling

Sample type	**Sample label**	**Sample type**	**Sample label**
…			

Date and signature of sample owner:

Date and signature of sample recipient:

Date and signature of sample recipient:

Date and signature of sample recipient:

Date and signature of sample recipient:

Annex 4 Test Cycle Report

Test Cycle Report

Treatment system: SiCURE, Siemens
Ship name: Horizon Hunter
Date and time for ballast water uptake: dd.mm.20xx, 20:03 - 20:17.

- Position of ship during beginning of ballast water uptake: region, ca. 28.5 N, 33.8 E.
- Distance travelled during uptake was ca. 14 nm.
- Water depth ca. 60 m.
- Distance to nearest main land ca. 8 nm.

Date and time for ballast water discharge: dd.mm.20xx, 20:40 - 21:42.

- Position of ship during beginning of ballast water discharge: ca. 29.1 N, 34.4 E.

Holding time of ballast water between uptake and discharge ca. 20 hours.
Weather conditions during the test: good, very little ship movements.

Water quality and number of organisms in uptake and discharge water

Parameter	**Unit**	**Uptake water[14]**			**Discharge water**						
		control	**before treatment**	**IMO**	**control**	**IMO[15]**	**treated**			**aver.**	**IMO**
							# 1	**# 2**	**# 3**	**#1-#3**	
Temperature	°C			-		-					-
Salinity	PSU			-		-					-
POC *	mg/l			-		-					-
TSS *	mg/l			-		-					-
Sample vol. >50 µm	Litres			>1000		>1000					>1000
Organisms >50µm	org./1m³			>90[16]		>10					<10
Sample vol. 50-10 µm	Litres			>1		>1					>1
Organisms 10-50µm*	org./1ml			>90[17]		>10					<10
Sample vol. bacteria	Litres			>0,5		-					>0,5
Escherichia coli	cfu/100ml			-		-					<250
Intestinal *Enterococci*	cfu/100ml			-		-					<100
*Vibrio cholerae***	cfu/100ml			-		-					<1

* Analyzed at NIOZ, Texel, The Netherlands. ** Analysed at IBEN, Bremerhaven, Germany.

Remarks: describe problems during sampling and sample analysis

Conclusions: test validity, compliance with D-2

Date and signature of the sampling crew leader

dd.mm.20xx S. Gollasch

><((((°>
GoConsult

[14] IMO does not require to sample the treated line during uptake (G8, Annex, Part 2, paragraph 2.2.2.6.2). However, the wording is unclear and consequently such samplings are recommended (see sampling protocol).
[15] IMO does not require the bacteriological analysis of the control discharge water (G8, Annex, Part 2, paragraph 2.2.2.5). Any weakness here will not influence the test validity.
[16] current version of G8, Annex, Part 2, paragraph 2.2.2.5 (see sampling protocol)
[17] current version of G8, Annex, Part 2, paragraph 2.2.2.5 (see sampling protocol)

Annex 5 Sampling event details, documentation at the sampling point

The following data need to be recorded during the ballast water sampling at the sampling point, e.g. in the engine room. The placeholders may be deleted and the tables be printed for onboard use.

Ballast water **uptake**

Date		
Sampling duration	start: end	
Ship position	Region	
Ship position	Lat/lon	
Distance to nearest land		
Water depth during the entire uptake event	from to m.	
Comment		
Sample type	Temperature	Water volume filtered
• control		
• treated		

Ballast water **discharge**
(R1 - R3 are the three replicate samplings required at the discharge of the treated water).

Date		
Sampling duration	start: end	
Ship position	Region	
Ship position	Lat/lon	
Comment		
Sample type	Temperature	Water volume filtered
• control		
• treated, R1		
• treated, R2		
• treated, R3		

Annex 6 Optional: Sampling team locator

As it is sometimes essential to arrange a meeting with all sampling team members at short notice the following may be printed and hang on the outside of the cabin door of each sampling team member. A magnet may be used to indicate where the team members are. This should be updated whenever a team member leaves the cabin.

Name of sampling team member

Please find me:

- here in my cabin, please come in
- here in my cabin, **please do only disturb in urgent cases!**
- in the cabin of the other GoConsult sampling team member
- in the cabin of ballast the water treatment system personnel
- in the engine room
- in a meeting at the ships office
- in a meeting with the sampling responsible ship's crew
- in the laboratory
- on the wheel house
- out for a meal
- on deck
- at the gym

Annex 7 Items needed for uptake and discharge samplings at the sampling point

This Annex should be printed and put on the wall in the cabin where the sampling gear will be packed.

Items to be brought to the sampling point:

Item	Number	
	uptake*	discharge**
Essential to bring		
Writing utensils (pencil, paper)	1 set	1 set
Tape (to avoid net damage/cuts from metal screws used to fit hose to flow meter inlet tube)	1	1
Bucket with lid	2	4
Thermometers (including digital version)	2	4
Measuring cups with wide opening (to take "continuous" drip sample)	2	4
Wash bottles	2	4
HydroBios plankton nets	2	4
Rope to hang plankton nets	2	4
HydroBios cod-ends (one as replacement)	3	5
HydroBios filtering socks (as replacement)	3	5
HydroBios inline flow meter[18] with pipes and valves	2	4
Replacement sealing rings for flow meters	4	8
Towels for cleaning	1	1
Optional to bring		
Hooks to hang plankton nets	2	4
1 L sample bottles with lids to store organism extracted from cod-end during longer sampling events	2	4
1 L sample bottles with lids to store water for bacteriological analysis	2	4
Big bags with water tight inlay to be used in case cod-ends become completely clogged	2	2

* During uptake one sample is taken from the control line and another from the treated line.
** During discharge one sample is taken from the control line and three from the treated line (as replicates).

It is further recommended to bring hearing protection, a towel and some drinking water for longer sampling events.

[18] To avoid the water supply hoses to disconnect or to slip off the flow meter inlet tube ensure that a double set of hose connecting screws are used.

Annex 8 Sample labelling

Except samples for organisms above 50 micron in minimum dimension, which will be analysed directly after sampling, each sample needs to be clearly labelled. The following is a labelling suggestion. The placeholders may be deleted and the tables be printed for onboard use.

TSS/POC
Write directly on Petri dish with waterproof pen. Secure both Petri disc plates with transparent tape. Record Petri disc number on *Sample event details and zooplankton analysis sheet* (Annex 2).

Uptake sampling

dd.mm.yy
Uptake
Volume filtered
Region (e.g. off Pacific Mexico)
Control

dd.mm.yy
Uptake
Volume filtered
Region (e.g. off Pacific Mexico)
Treated

Discharge sampling

dd.mm.yy
Discharge
Volume filtered
Region (e.g. off Pacific Mexico)
Control

dd.mm.yy
Discharge
Volume filtered
Region (e.g. off Pacific Mexico)
Treated, R 1

dd.mm.yy
Discharge
Volume filtered
Region (e.g. off Pacific Mexico)
Treated, R 2

dd.mm.yy
Discharge
Volume filtered
Region (e.g. off Pacific Mexico)
Treated, R 3

Organisms less than 50 and bigger than 10 micron in minimum dimension

Use self-glue paper stickers, write with pencil. Place sticker on the side of the sampling bottle and secure with transparent tape. In case the PAM-fluorometry is used onboard no living samples need to be stored.

Uptake sampling

Living Lugol preserved

dd.mm.yy	dd.mm.yy
Uptake	Uptake
Volume filtered	Volume filtered
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Control	**Control**
Measure volume!	Measure volume!

dd.mm.yy	dd.mm.yy
Uptake	Uptake
Volume filtered	Volume filtered
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated	**Treated**
Measure volume!	Measure volume!

Discharge sampling

Living Lugol preserved

dd.mm.yy	dd.mm.yy
Discharge	Discharge
Volume filtered	Volume filtered
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Control	**Control**
Measure volume!	Measure volume!

dd.mm.yy	dd.mm.yy
Discharge	Discharge
Volume filtered	Volume filtered
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 1	**Treated, R 1**
Measure volume!	Measure volume!

dd.mm.yy	dd.mm.yy
Discharge	Discharge
Volume filtered	Volume filtered
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 2	**Treated, R 2**
Measure volume!	Measure volume!

dd.mm.yy	dd.mm.yy
Discharge	Discharge
Volume filtered	Volume filtered
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 3	**Treated, R 3**
Measure volume!	Measure volume!

E. coli
Write directly on Petri disc with waterproof pen.

Take a note and place this on your door or anywhere you see it frequently when incubation time terminates and the bacteriae dishes need to be analysed.

Uptake sampling

dd.mm.yy	dd.mm.yy	dd.mm.yy
Uptake	Uptake	Uptake
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Control	**Control**	**Control**

dd.mm.yy	dd.mm.yy	dd.mm.yy
Uptake	Uptake	Uptake
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated	**Treated**	**Treated**

Discharge sampling

dd.mm.yy	dd.mm.yy	dd.mm.yy
Discharge	Discharge	Discharge
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Control	**Control**	**Control**

dd.mm.yy	dd.mm.yy	dd.mm.yy
Discharge	Discharge	Discharge
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 1	**Treated, R 1**	**Treated, R 1**

dd.mm.yy	dd.mm.yy	dd.mm.yy
Discharge	Discharge	Discharge
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 2	**Treated, R 2**	**Treated, R 2**

dd.mm.yy	dd.mm.yy	dd.mm.yy
Discharge	Discharge	Discharge
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 3	**Treated, R 3**	**Treated, R 3**

Enterococci
Write directly on Petri disc with waterproof pen.

Take a note and place this on your door or anywhere you see it frequently when incubation time terminates and the bacteriae dishes need to be analysed.

Uptake sampling

dd.mm.yy	dd.mm.yy	dd.mm.yy
Uptake	Uptake	Uptake
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Control	**Control**	**Control**

dd.mm.yy	dd.mm.yy	dd.mm.yy
Uptake	Uptake	Uptake
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated	**Treated**	**Treated**

Discharge sampling

dd.mm.yy	dd.mm.yy	dd.mm.yy
Discharge	Discharge	Discharge
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Control	**Control**	**Control**

dd.mm.yy	dd.mm.yy	dd.mm.yy
Discharge	Discharge	Discharge
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 1	**Treated, R 1**	**Treated, R 1**

dd.mm.yy	dd.mm.yy	dd.mm.yy
Discharge	Discharge	Discharge
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 2	**Treated, R 2**	**Treated, R 2**

dd.mm.yy	dd.mm.yy	dd.mm.yy
Discharge	Discharge	Discharge
1 ml	10 ml	100 ml
Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)	Region (e.g. off Pacific Mexico)
Treated, R 3	**Treated, R 3**	**Treated, R 3**

Cholera
Write directly on sample jar with waterproof pen. As the samples need to be shipped and the incubator may need to be opened at customs or security, do not write "Cholera" on any of the samples as this may cause trouble. Instead write C-water samples.

Uptake sampling

dd.mm.yy
Uptake
100 ml
Region (e.g. off Pacific Mexico)
Control

dd.mm.yy
Uptake
100 ml
Region (e.g. off Pacific Mexico)
Treated

Discharge sampling

dd.mm.yy
Discharge
100 ml
Region (e.g. off Pacific Mexico)
Control

dd.mm.yy
Discharge
100 ml
Region (e.g. off Pacific Mexico)
Treated, R 1

dd.mm.yy
Discharge
100 ml
Region (e.g. off Pacific Mexico)
Treated, R 2

dd.mm.yy
Discharge
100 ml
Region (e.g. off Pacific Mexico)
Treated, R 3

Annex 9 Set-up of sampling gear at the sampling point

The following outlines the working steps to take before the sampling may start, during sampling and after the sampling was completed.

This Annex should be formatted in font size 14, printed and hang to the wall at the sampling point.

Before the sampling starts

- Identify control and treated sampling line (ask vendor personnel)
- Place labelled buckets near sampling line outlets
- Place one thermometer at the bottom of each bucket
- Put wash bottle next to each bucket (check label)
- Put measuring cup to collect "continuous" drip sample next to each bucket
- Hang up HydroBios nets with ropes over water collectors
- Unscrew lid of cod-ends, put lid next to bucket (check label on lid) and screw cod-end to net
- **Check if cod-end valve is closed completely**
- Make sure that flow meter sealing rings are fitted and don't fell off
- Hang flow meters with top outlet tube extending into net and secure flow meter to net with rope
- **Check if flow meter was put back to zero** (if not, open transparent lid and press the left two bottoms simultaneously for two seconds)
- Check if flow meter inlet tube is properly fixed to sampling line, but don't connect yet with flow meter.
- When manipulating the flow meter valve substantial pressure may occur in the sampling line. Therefore it is preferred to have a double fixing ring screwed to the sampling line and flow meter inlet connection.
- Use tape to ensure that the metal fixing ring and screw cannot damage the net
- Note date and sample type

During sampling

- After the water flow has started wait at least 2 minutes with the open flow meter valve (at the flow meter inlet tube) to wash the pipes. When doing so drain water into water collecting buckets underneath nets
- Check if the discharge of the water collecting buckets works
- Note start time of the sampling event
- Close flow meter valve completely and connect to flow meter
- Open valve and start feeding water into net. Minimize water spillage to the floor, i.e. make sure that the filtered water enters into the water collecting buckets underneath the net. If needed manipulate the position of the outlet tube of the flow meter to minimize water spillage
- Fill wash bottle with water filtered through net and put bottle into bucket
- Ask vendor personnel for likely duration of the sampling event and roughly calculate the frequency of measuring cups needed to fill bucket[19].
- Tell vendor personnel to give a 10 minute warning before the sampling event should be terminated

[19] Be prepared that the estimated duration of the sampling event is inaccurate. As a result make sure that you "oversample" in the beginning to guarantee that the 5 L are in the bucket when the sampling event comes to an end. This may be done either by a higher sampling frequency in the beginning or by filling the measuring cup more in the beginning and reduce the volume sampled towards the end.

- Fill the measuring cup every few minutes with water from flow meter outlet and empty into bucket. Put lid back on bucket to avoid possible water spillage from neighbouring sampling line or dust etc to get into bucket. After sampling is completed at least 5 L of water should have been collected over the entire sampling time[20]
- During longer sampling events, i.e. more than 30 minutes, stop the water supply every 10 minutes and extract the concentrated organisms from the cod-end into a 1 L bottle to avoid organism damage during sampling. When doing so carefully unscrew cod-end that only the upper connection (cod-end to net) opens and use the tap at the cod-end to drain organism into 1 L bottle
- Control and manipulate water flow with flow meter valve to avoid water collectors under the net to overflow. However, make sure that at least 1000 L of water are filtered through the net
- Measure water temperature in all buckets after thermometer was at least 10 minutes covered with water and record temperature

End of sampling

- Close valve of flow meter, disconnect inlet tube from flow meter, place it in water collector and open valve again
- Record end time of sampling
- Use wash bottle to clean net and put wash bottle back into bucket
- Carefully unscrew cod-end that only the upper connection (cod-end to net) opens
- Place cod-end carefully into bucket thereby allowing water to drain into cod-end via the filtering panels. Make sure that no sample is spilled into bucket.
- In case the cod-end was emptied into a 1 L bottle during longer sampling events, add water to the 1 L bottle to maximum level to give the concentrated organisms more room
- Screw cod-end lid on and drop cod-end into bucket
- Fill 1 L bottle for bacteriae analysis and put in bucket
- Put lid on bucket and make sure lid sits tight
- Read and note water volume filtered from each flow meter
- Put flow meters into transport bag
- Take nets down and put back into net plastic bag and put all nets into transport bag
- Take ropes and hooks down and put into transport bag
- Carry all items back to cabin / laboratory

[20] This water is used to measure TSS/POC, salinity, phytoplankton and bacteriae.

Annex 10 Sample transport reminder

Print this page and hang to the wall where you see it before you leave the vessel.

Take all samples from fridge and freezer with you.

- TSS/POC
- Phytoplankton living
- Phytoplankton preserved

After packing Styrofoam boxes, seal box with tape.

Annex 11 Time line for bacteriae analysis

Print this page and hang to the wall where you see it frequently.

Test run number:

Start of bacteriae incubation of **uptake samples**: day, hour

Bacteriae	Temperature	Incubation duration	Inspection time (add day and time)
Cholera	37 °C	until inspection	done later on land
Enterococci	37 °C	48 hrs	day: time:
E. coli	37 °C	24 hrs	day: time:
E. coli (yellow "suspects")	44 °C	24 hrs	day: time:

Start of bacteriae incubation of **discharge samples**: day, hour

Bacteriae	Temperature	Incubation duration	Inspection time (add day and time)
Cholera	37 °C	until inspection	done later on land
Enterococci	37 °C	48 hrs	day: time:
E. coli	37 °C	24 hrs	day: time:
E. coli (yellow "suspects")	44 °C	24 hrs	day: time:

Annex 12 DNV Audit confirmation of GoConsult onboard work

DET NORSKE VERITAS

DET NORSKE VERITAS AS
Approval Ship and Offshore
Cargo Handling and Piping Systems
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http://www.dnv.com
Org. No: NO 945 748 931 MVA

GoConsult
Hamburg

Att.: Stephan Gollasch

Your ref.:	Our ref.:	Date:
	NACNO880/JADMO/BWMS Correspondence-J-55	2010-09-17

Confirmation of surveys of shipboard tests of ballast water treatment system

In connection with the Type Approval of the Unitor Ballast Water Management System according to the G8 Guidelines of the Ballast Water Management Convention, DNV surveyor attended the shipboard tests on the MV Toronto between 6th and 14th June 2010.

The QAPP of the shipboard tests as well as the QMP of GoConsult were approved by DNV prior to attendance of the DNV surveyor.

GoConsult, represented by

- Stephan Gollasch, PhD, senior scientist, marine biologist, GoConsult, Hamburg
- Matej David, professor for maritime studies and transport, for GoConsult, Hamburg

performed the complete sampling during the tests and part of the analysis which was their responsibility according to the QAPP.

The following was done:

1 SURVEY OF THE SAMPLING PROCEDURES

DNV surveyor went through the sampling procedures together with sampling leader Dr. Stephan Gollasch and Professor Matej David.

1.1 Gear check

DNV Surveyor received information on the use of incubators, microscope, electronical equipment like salinity meter etc, and PAM and flow metres to measure the sampling volume.

The PAM is calibrated by comparison with the PAM at NIOZ in the Netherlands; the flow meter is a propeller type.

1.2 Checklists

Important checklists as given in different appendices in the QAPP are hanged on the walls of the analysis rooms and at the sampling site in the engine room.

Dr. Gollasch also showed the DNV surveyor a record book in paper format that will accompany him when noting the different parameters during sampling.

1.3 Sampling and transfer procedures

Both Dr. Gollasch and Dr. David perform the sampling, pack and transport the samples for analysis. Organisms between 10µm and 50µm are analysed by the Flowcam by Anja Terörde and are not in the scope of GoConsult.

DET NORSKE VERITAS

1.4 Labelling

Dr. Gollasch and Dr. David showed DNV surveyor how all parts used for one replicate (treated and control) are clearly labelled.

The same labels (R1, R2 for treated and C for control) are going to be used on sampling pipes.

Upon question on anti-contamination from DNV surveyor, Dr. Gollasch and Dr. David explained that the sampling drums will be clearly placed away from each other and with the labelling being as explained above, there will not be any mixing of samples.

1.5 Storage

No storage of samples is being done so retrieval of data and samples is not possible.

1.6 Transportation to land for lab analysis

Dr. Gollasch informed that the Phytoplankton are cooled down and can be analysed after maximum 10 days.

For the water quality parameters like TSS etc. a deep freezer is used until the samples reach a lab.

For the cholera, it is stored in an incubator that is plugged to a normal power outlet and heated. This is also done through a power connector in a car.

The protocol for sample transfer as given in the QAPP is followed.

2 SURVEY DURING TESTS

2.1 Surveys of test 1

Sampling was done in a satisfactory manner.

2.2 Surveys of test 2

Sampling was done in a satisfactory manner.

The first part of the analysis done by Dr. Gollasch and Dr. David were done according to the approved QAPP.

2.3 Surveys of test 6

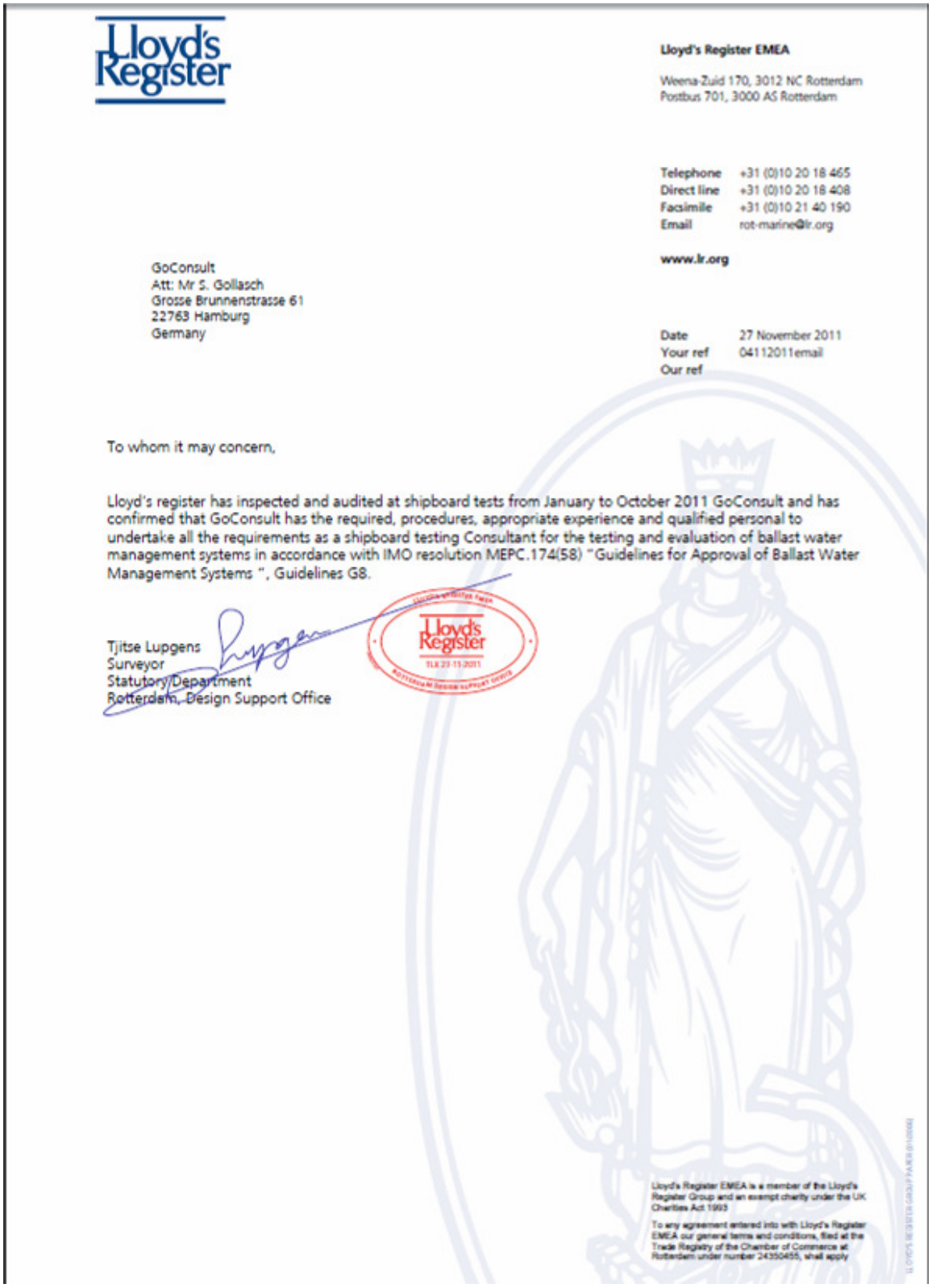
The final part of the analysis done by Dr. Gollasch and Dr. David were done according to the approved QAPP.

Yours faithfully
for DET NORSKE VERITAS AS

Øivin Lorentzen
Head of Section
Cargo Handling and Piping Systems

Jad Mouawad
Senior Engineer/Surveyor

Annex 13 Lloyds Register Audit confirmation of GoConsult onboard work



Lloyd's Register

Lloyd's Register EMEA

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Email rot-marine@lr.org

www.lr.org

GoConsult
Att: Mr S. Gollasch
Grosse Brunnenstrasse 61
22763 Hamburg
Germany

Date 27 November 2011
Your ref 04112011email
Our ref

To whom it may concern,

Lloyd's register has inspected and audited at shipboard tests from January to October 2011 GoConsult and has confirmed that GoConsult has the required, procedures, appropriate experience and qualified personal to undertake all the requirements as a shipboard testing Consultant for the testing and evaluation of ballast water management systems in accordance with IMO resolution MEPC.174(58) "Guidelines for Approval of Ballast Water Management Systems ", Guidelines G8.

Tjitse Lupgens
Surveyor
Statutory Department
Rotterdam, Design Support Office